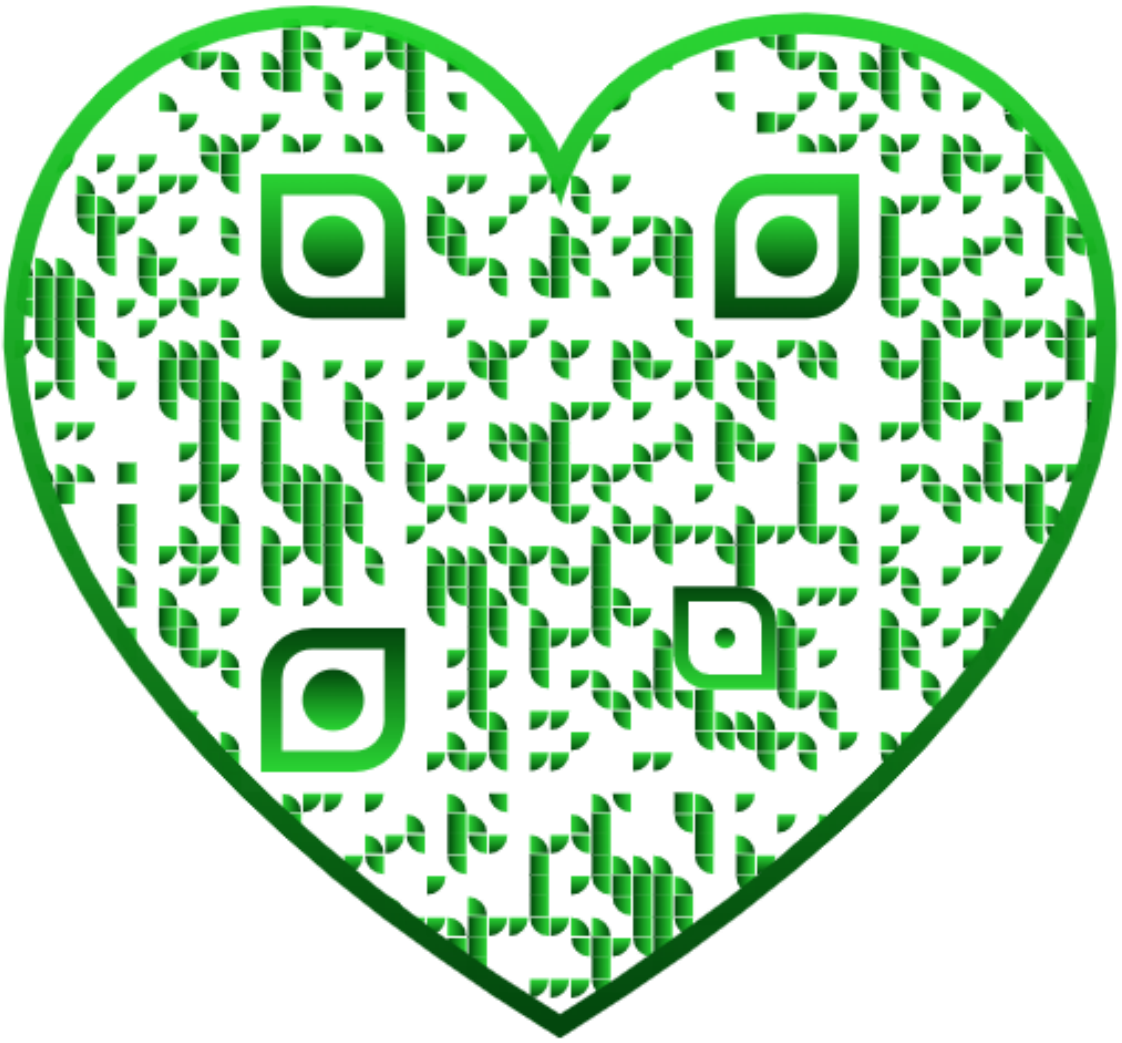
LIMIT TEST



- Impurities in Pharmaceuticals are the **unwanted chemicals** that remain with
  - the active pharmaceutical ingredients (APIs),
  - developed during formulation,
  - up on aging of both API and formulated APIs to medicines
- The presence of these unwanted chemicals even in smaller amounts may influence the **efficacy and safety** of the pharmaceutical products
  - **identifying** and **quantifying** of impurity in the pharmaceuticals, is now getting critical attention from **regulatory authorities**
- Pharmacopoeias incorporate limits to allowable levels of impurities present in APIs or formulations

- Chemical purity is **freedom** from foreign matter
- It is virtually **impossible** to have absolutely pure chemical compound
- The level of purity of the pharmaceutical substance depend partly on the **cost effectiveness** of the **methods** of purification and **stability** of the final product
  - **Purification** of chemical cpd is a **very expensive** process, hence one has to strike a balance in order to obtain a pharmaceutical substance at **reasonable cost** yet **sufficiently pure** for all pharmaceutical purposes

Definition:

- **Limit tests** are **quantitative** or **semiquantitative tests** designed to **identify** and **control** small quantities of impurity which are **likely to be present** in drug samples
- **Pharmacopoeias fix the limits of tolerance** for these impurities
- The governing factor for these tests is to determine **how much** impurity is likely to **be harmful**, or to bring about **technical and other difficulties**, when the substance is used.

- The method can be either general or specific:

1. General limit test

- **Clarity of solution**
  - Solutions for injections should be reasonably free from particulate matter.
- **Color of solution**
- **Moisture,**
- **Volatile matter and**
- **residual solvents**

2. Limit tests for specific impurities

- Considerable emphasis is placed on the limitation of physiologically harmful impurities.

- **Limit Tests for Metallic Impurities**

  E.g. The control of toxic elements (As, Sb, Pb, Cd, Hg)

☞Specific tests for acid radical impurities

- The most common acid radical impurities are chloride & sulphate
- Also applied for a number of other acid radical contaminants
  - Cyanide
  - Oxalate
  - Phosphate
  - Silicate

☞ Limits on ash values

- The ash values usually represent the inorganic residue present in official herbal drugs and pharmaceutical substances.
- These values are categorized into four heads:

  (a) Ash Value (Total Ash)

  (b) Acid-Insoluble Ash

  (c) Sulphated Ash

  (d) Water-Soluble Ash

i. Ash Value (Total Ash)

- Ash value normally designates the presence of inorganic salts
  - E.g., calcium oxalate found naturally in the drug,
  - Inorganic matter derived from external sources

ii. Acid-Insoluble Ash

  - Designed to measure the amount of ash insoluble to diluted hydrochloric acid.
    - By treating the 'total ash' with acid

- comparatively forms a better test to detect and limit excess of soil (silica) in the drug than does the ash

iii. Sulphated Ash

- Specifically for the **synthetic organic compounds**, the Pharmacopoeia prescribes values for sulphated ash
- The sulphated ash is determined by igniting the substance with concentrated sulphuric acid. e.g.
  - Activated Charcoal, NMT : 5.00%
  - Griseofulvin, NMT : 0.10%
  - Ascorbic acid, NMT: 0.1%

iv. Water-Soluble Ash

- Water-soluble ash is specifically useful in detecting such samples which have been extracted with water.

- A typical example of an official drug is that of 'Ginger', the water-soluble ash of which is found to be not more than 6.0%.

iv. Limits on moisture content

- Many official drugs contain varying amount of water either in the form of crystallization i.e. hyadrates or in absorbed form.
- Moisture plays remarkable negative role in pharmaceutical product, particularly for solid dosage forms.
- Both physical and chemical stability of some drugs are affected by moisture.
- Moisture is absorbed on the surface of solid drugs and increases the rate of decomposition, causes agglomeration and dissolution of drugs.

- Presence of moisture possesses a critical challenge on drug stability.
  - Moisture accelerates:
    - The hydrolysis of drug
    - Facilitates reaction with other excipients,
    - Thereby affecting stability and shelf life of the final product.
  - Therefore, it is important to specify limits of moisture content.
- **Methods of moisture content determination**
  - Loss on drying method
  - Azeotropic distillation method
  - Karl- Fischer titration method
  - Other methods

A. Loss on drying method

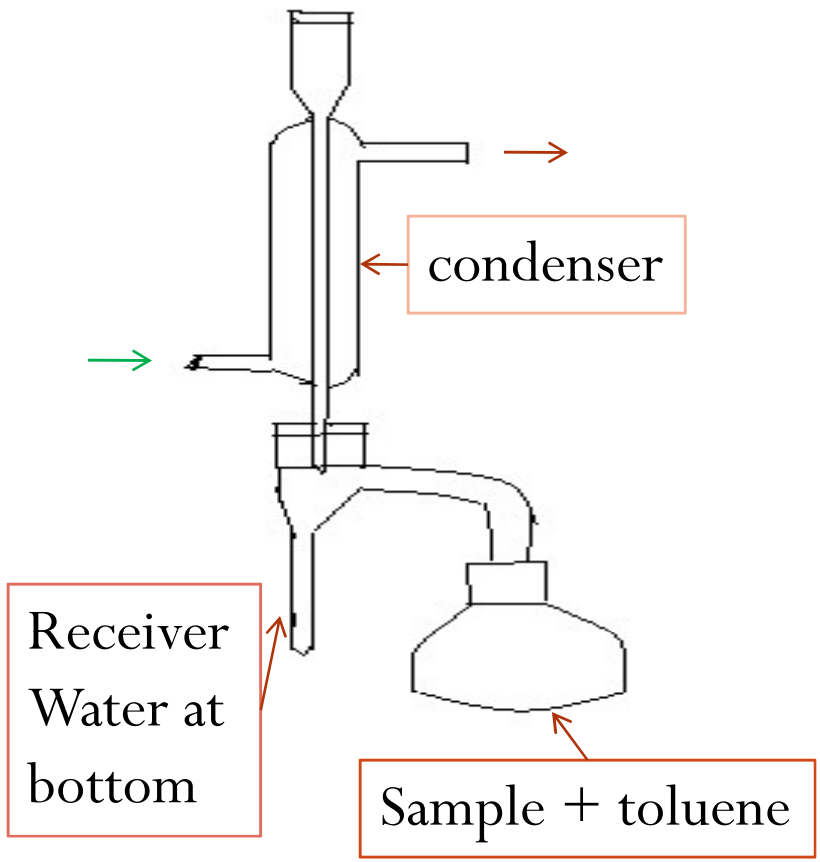
- It is the loss of weight in % w/w resulting from water & volatile matters that are lost under specified conditions.
- Reflects the net weight of a pharmaceutical substance being dried at a specified temp either at an atmospheric or under reduced pressure
- Advantages
  - Easy to use, Relatively rapid,
  - Many samples can be analyzed simultaneously
- Disadvantages
  - Destructive

B. Azeotropic distillation method

- A binary solution with the composition of the constant-boiling mixture will distill as if it were a single substance
  - i.e, without change in boiling point or composition
- such a constant-boiling mixture is called an azeotrope
- This phenomenon has been used to advantage for separating water from other components of a mixture
- **Water** and **toluene** form an azeotrope with bp 84.1 ^{0}c
  - The azeotrope contains 19.6% water

- Since these two liquids are practically immiscible, the condensed distillate separates in to two layers

- The volume of the aqueous (lower) phase is measured to give a direct measure of the water content of the sample
- the vapors rise in to the condenser, and the distillate falls in to the receiver, where it separates in to two phases



• forms an azeotropic vapor phase with water in the distillation at a temperature <100 °C

C. Karl Fischer Method

- The Water Determination Test (KF Method) is designed to determine water content in substances,
  - Utilizing the quantitative reaction of water with KF reagent.
    - KF reagent which is composed of iodine, sulphur dioxide, pyridine and methanol.
    - Water present in the analyte reacts with the KF reagent as shown below:

$$H_2O + I_2 + SO_2 + 3C_5H_5N \longrightarrow 2(C_5H_5\overset{+}{N}H)I^- + C_5H_5N.SO_3$$

$$C_5H_5N.SO_3 + CH_3OH \longrightarrow (C_5H_5\overset{+}{N}H)O^-SO_2.OCH_3$$

•The composition of the reagent should be

$1I_2 : 1SO_2 : 1CH_3OH : 3C_5H_5N$

- In first step, the oxidation of sulphur dioxide takes place by iodine to yield **sulphur trioxide** and hydrogen iodide thereby consuming one mole of water.
  - i.e. one molecule of iodine disappears against each molecule of water present in the given sample.
  - There are two determination methods:
    - Volumetric titration method
    - Coulometric titration method

1. Volumetric titration method

- Water content is determined
  - By measuring the amount of iodine consumed as a result of reaction with water in a sample,
    - From the burette volume of the iodine-containing KF solution
    - Water as a major component: 100 ppm - 100 %

2. Coulometric titration method

- First, iodine is produced by electrolysis of the reagent containing iodide ion, and then, the water content in a sample is determined
  - By measuring the quantity of electricity which is required for the electrolysis (i.e., for the production of iodine)
  - Water in trace amounts: 1 ppm - 5 %

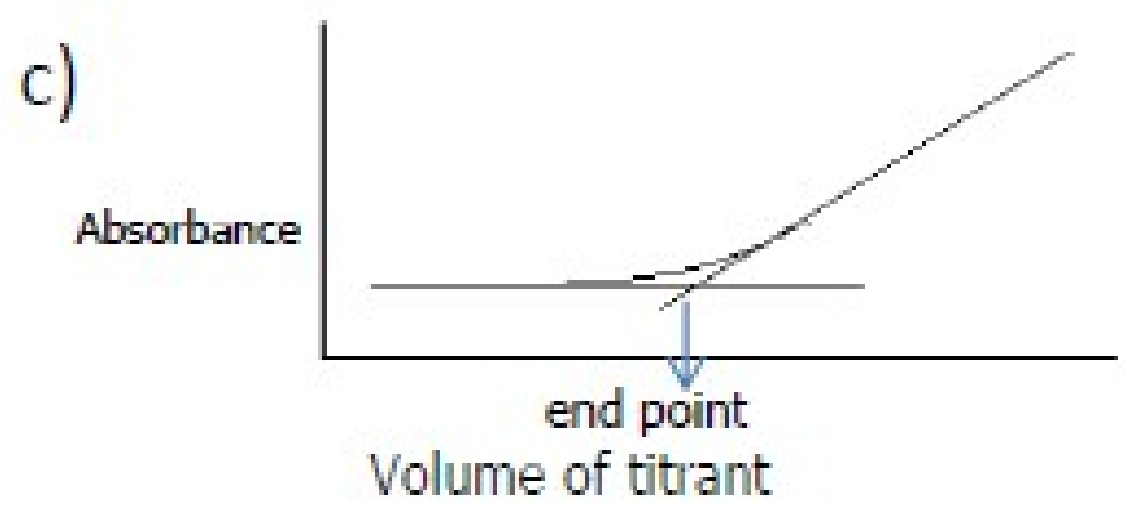
- The end point in a Karl Fisher titration can be located in several ways.

  - Visual detection

    - Before the end point the solution is yellow
    - After the end point the first excess of the reagent imparts the characteristic brown I_2 color

Photometric method

- The active reagent absorbs light at 525nm
- Before the end point the absorbance remains close to zero
- After the end point, the absorbance increases linearly with volume of reagent.
- The end point marked by the intersection of the two straight lines.



V. Limit tests for metallic impurities

- The official compendia lay a great deal of emphasis on the control of physiologically dangerous, cumulative poisonous and harmful impurities, Such as lead, arsenic, mercury and iron present in pharmaceuticals
- All prescribed tests for impurities in the Pharmacopoeia usually fix certain limits of tolerance.
- By changing them into detectable derivatives/ compounds which can be easly compare with the standards.
  - Eg color and turbidity comparison with standards.

The end!